

## SEMICONDUCTOR NANOCRYSTAL-BASED CELLULAR IMAGING

### REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Application No. 60/335,521 which was filed on Oct. 31, 2001 and is hereby incorporated by reference in its entirety.

### STATEMENT OF GOVERNMENT SUPPORT

[0002] This invention was made during work partially supported by U.S. Department of Defense Award No. DAMD17-98-1-8182, DOD Advanced Research Projects Agency (DARPA) under Grant No. N00014-99-1-0728, and the National Institutes of Health under Grant No. 1 R01 RR-14891-01 through the U.S. Department of Energy under Contract No. DE-AC03-76SF00098. The government has certain rights in this invention.

### BACKGROUND OF THE INVENTION

#### [0003] 1. Field of the Invention

[0004] This invention generally relates to the field of semiconductor nanocrystals and to the field of cellular behavior and phenomena. More specifically, the invention provides a simple, reliable method of phagokinetic tracking using semiconductor nanocrystals. The invention has further application in the fields of observing live cell motility, migration, metastatic potential, cellular uptake and tracking cell lineage using semiconductor nanocrystals.

#### [0005] 2. Description of the Related Art

[0006] Metastasis of cells is a major problem in cancer. Migration of cancerous cells leads to metastases and the formation of secondary tumors. Studies of chemotherapeutic agents depend upon cell motility assays. Current assays are cumbersome and prone to error, and require killing the cells which prevents further analyses.

[0007] The most direct method for observing cell motility currently is time lapse videos of cells in culture as described by Rajah, et al., *In vitro cell. Dev. Biol.—Animal* 34, 626-628 (1998). However, this method is restricted to measurements on just a few cells at a time, and therefore this approach is not widely used to make statistically significant studies of cell populations. Improved statistics can be obtained with the "scratched wound method" in which a region of the cell culture substrate is denuded of cells, and then the time scale for the filling of this "hole" is observed; unfortunately, the history of the cell migration paths are lost, and the analysis is complicated by subjective analysis of the complex and variable patterns of the cell motion that lead to hole filling. Környei, et al. *J. Neurosci. Res.* 61, 421-429 (2000); R. R. Bürk, *PNAS* 70, 369-371 (1981). A significant advance occurred with the development of the Boyden Chamber invasion assay, in which cells are seeded on one side of a membrane, and the rate of appearance of cells on the other side is monitored. Yao, et al., *J. Neurosci. Res.* 27, 36-42 (1990); S. Boyden, *J. Exp. Med.* 115, 453-466 (1962).

[0008] There are many commercially available versions of the Boyden Chamber technology. Many companies have modified the Chamber technology to use, for example, a stainless steel chamber (e.g. Neuro Probe BY312 BOYDEN

CHAMBER, made by Neuro Probe, Inc., Gaithersburg, Md.) or creating chambers by clamping glass cover slips with silicon spacers (e.g. Hemogenix MODIFIED BOYDEN-CHAMBER #0729.000, made by HemoGenix LLC, Irmo, S.C.), or plastic microplates wherein each of the wells has a coated membrane on the bottom for cells to migrate through (e.g. QCM™ Quantitative Cell Migration Assay, made by CHEMICON International, Inc., Temecula, Calif.). The Boyden Chamber method is by far the most widely used, yet it is laborious; most protocols require that the cells be fixed or stained, and thus destroyed, and do not allow for real time variation of the external condition. Cells can be quantitated through various means such as by optical density or by fluorescence microscopy, but a significant number of cells are frequently lost during processing which decreases accuracy.

[0009] A recent cell motility assay has been developed and patented by Biometric Imaging, Inc. See Jarnagin, et al., U.S. Pat. No. 6,238,874. Jarnagin et al. describe an apparatus and method for assaying motility in response to a chemotactic agent. The apparatus provides a chamber having two regions. The apparatus facilitates the establishment of a concentration gradient of the chemotactic agent which increases on progressing from the first region to the second region, also called the interrogation region. The individual cells are detected by comparing the distribution of detected, position-assigned cells in the interrogation region at two or more time intervals. The method of using this apparatus involves labeling the selected type of cell with a fluorescent compound and detecting the peak fluorescence of individual cells or a population of cells. The positional information for each cell or a population of cells is detected over a period of time by tracking the fluorescence and storing the data as pixel images of coordinates.

[0010] Albrecht-Buehler proposed a method for studying cell motility based upon observations of "phagokinetic tracks." G. Albrecht-Buehler, *Cell* 12, 333-339 (1977); G. Albrecht-Buehler, *Cell* 11, 395-404 (1977). In a most general way, a phagokinetic track is generated when a cell passes over a layer of "markers", and ingests them, leaving behind a blank spot equal to the area the cell has traversed. In principle the method is very powerful, as it provides a rapid and automatic method for integrating cell motility while preserving the history of individual paths. Until now, the method has only been used in a limited way due to problems with the available markers. Markers such as sub-micron Au (gold) particles, India ink or latex particles have been used because they can be imaged optically using darkfield microscopy. However, these markers also impose many limitations so that the technique has not received widespread acceptance.

[0011] The Au particles used in the phagokinetic tracking assay must be large (0.15 microns) in order to be observable optically. Such large particles do not stick well to the substrate, and therefore have to be grown directly on the substrate in a process that yields highly inhomogeneous particle distributions. Since the particles are grown directly on the substrates, by pouring a hot (near boiling) aqueous solution onto the substrate, the range of usable substrates is limited. Further, due to the large size of the Au microcrystals, when a cell moves one diameter, it ingests a volume of Au corresponding to ~1% of the total cell volume, and there is a strong possibility that this perturbs the cell motility. This